Vesicular Stomatitis Virus in the Treatment of Hepatocellular Carcinoma

A Senior Project

presented to

the Faculty of the Animal Science Department

California Polytechnic State University, San Luis Obispo

In Partial Fulfillment

of the Requirements for the Degree

Bachelor of Science

by

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I heard about this internship at Mt. Sinai Medical Center in Manhatten, NY from my cousin who is a program director in the Department of Oncological Sciences. Even though this program was geared toward students interested in attending medical school, I saw value in the research experience this internship could provide me with.

I was placed in the Oncological Sciences department, located on the 13th floor of the Icahn Building. My mentor was Dr. Miriam Merad, MD, PhD, although most of my work was on a project in Dr. Woo's lab. The reason for this is due to the amount of animal work involved in the project for Dr. Woo's lab. Being that I am interested in veterinary medicine, the researchers were willing to let me help on their experiments that involve any sort of animal work, so I floated among the technicians and different labs on the floor. This allowed me to gain experience with many different animal procedures, including parabiotics with mice, gavage, hepatic artery injections, suturing, portal vein injections, and other animal protocols. All of these experiences are applicable to a career in veterinary medicine and have really opened my eyes to what I can do with an Animal Science degree or Doctorate of Veterinary Medicine besides being a general practitioner. The project that I was involved in the most is looking at using an oncolytic virus, specifically vesicular stomatitis virus (VSV), as a treatment option for patients with hepatocellular carcinoma (HCC).

On a daily basis, I gathered and recorded body weights on a triple beam balance and recorded observations on the general health and appearance of the rats, looking for signs of neurotoxicity. Since this treatment is going to the FDA, everything from the health of the rats to the lot number on the isoflourane must be recorded on standard sheets. I calculated the dosage of IFN alpha depending on the weight of the animal, set-up the procedural area, drew up the correct amount in the syringe, and helped restrain the animals. What I learned while doing this procedure several times is that the warmer the water is for the tails to soak, the more apparent the vein and artery became and the easier the IV injections were. For the procedure with the second group of ten rats, I was taught and eventually allowed to prepare the surgical site by shaving and sterilizing the abdominal area. I opened the rats via a midline laparotomy (~3 cm long) and prepared the area for the procedure. After the virus was injected into the hepatic artery, I sutured the rats closed and gave a subcutaneous analgesic injection and ear tagged each rat with their appropriate identifying number in case the tail identification wore off.

Unfortunately, I was not able to see the project to completion as the results from pathology take several months to come back to the researchers. From the research I conducted and talking to the researchers conducting the study, the results from this current study should be more effective than the previous study that was conducted where interferon alpha was not added. The following paper presents the material to the reader and the data tables were taken from the previous study conducted by the same group of researchers. It can be assumed the results would be better than the presented data.

ABSTRACT

Hepatocellular carcinoma (HCC) is a type of liver cancer common in adults that accounts for over one million cases annually (Altomonte et al., 2009). A lack of successful, conventional treatment options has sparked an interest in using oncolytic viruses. The most successful and popular virus being studied currently is vesicular stomatitis virus (VSV). Vesicular stomatitis virus is a relatively non-pathogenic, negative-stranded RNA virus that can preferentially replicate in malignant cells and less so in normal cells (Barber, 2004). The virus's short life cycle allows for frequent and quick replication before the host cells antibodies begin to do their job and destroy the virus. Interferon alpha is a key aspect of the innate immune response and VSV happens to be particularly susceptible to it. Tumor cells have a defective interferon pathway, which allows the virus to replicate quickly and cause necrosis. The healthy cells are able to defend themselves and destroy the virus. In this study, the combination of a recombinant VSV and interferon alpha are used to treat rats with HCC. Hopefully the two used in conjunction can provide an effective treatment to patients with this disease.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a type of liver cancer common in adults that accounts for over one million cases annually (Altomonte et al., 2009). The potentially curative treatment options that are available to patients today are liver transplantation and surgical resection, though these treatments can only be applied to a small number of patients (Marozin et al., 2010). Other local treatments that can be applied to many patients have been shown to have frequent recurrence and poor long-term survival (Marozin et al., 2010). This lack of successful treatment options has sparked an interest in using oncolytic viruses.

The most successful and popular virus being studied currently is vesicular stomatitis virus (VSV). VSV is a member of the Rhabdoviridae family, genera Vesiculovirus and a relative to the rabies virus genera Lyssavirus. The virus is bullet shaped in structure and encodes for only five proteins. Vesicular stomatitis virus is a relatively non-pathogenic, negative-stranded RNA virus that can preferentially replicate in malignant cells and less so in normal cells (Barber, 2004). The virus's short life cycle allows for frequent and quick replication before the host cells antibodies begin to do their job and destroy the virus. This virus is usually asymptomatic in humans, while production animals such as cattle and swine can be non-lethally infected and develop lesions on their mucosal membranes (Barber, 2004). A benefit to using this particular virus in the treatment of cancer is that vesicular stomatitis virus is not endemic to North America, meaning there should be no preexisting neutralizing antibodies or memory cell immune responses to interfere with its replication potential (Altomonte et al., 2009).

VSV AS AN ONCOLYTIC VECTOR

To better understand how VSV can replicate in tumor cells and cause necrosis, the body's innate immune response and how that protects the host from infection must be understood. The first line of defense against an invading pathogen is the innate immune response. The synthesis and secretion of Type 1 interferons (IFN) alpha and beta, are a key aspect of this response. Interferons are known to exert potent antitumor, anti-viral, and immunomodulatory activities (Barber,

2004), which is key in protecting the host. Two events are required to trigger an effective anti-viral innate immune response: 1) detection of the invading virus by immune system receptors; and 2) initiation of protein signaling cascades that regulate the synthesis of IFNs (Seth et al., 2006). While it is not quite clear how induction of the IFN-alpha promoter is regulated or induced, it is possibly done via three different pathways. What is known, however, is that these three pathways converge on TRAF3 (a protein in the cytoplasm), which induces interferon production through the activation of other protein complexes (Pietras et al., 2006). IFN alpha and beta are synthesized by most cell types and share a common receptor (Balachandran et al., 2000), so they are readily available in the body.

Once IFN alpha/beta is produced, it induces the production of protein kinase RNA-activated (PKR), which is a component of the host's defense mechanisms, designed to restrict viral replication. The function of PKR is to inhibit virus translation to buy time for other members of the innate immune response, such as neutralizing antibodies, to be produced and strengthen the antiviral state. It buys time by interacting with a double-stranded RNA (dsRNA), which causes PKR to autophosphorylate and to catalyze the phosphorylation of substrate targets (Balachandran et al., 2000). The best example being a reduction in protein synthesis rates in the cell. With a reduced amount of protein being made, the cell cannot function at a normal level and will affect any body system that needs that protein to function.

Another way the alpha and beta interferons aid the immune response is through virus-induced apoptosis of cells. Cell surface death receptors such as FAS/CD95, a member of the tumor necrosis factor receptor (TNFR) family, are ligated. This ligation leads to the recruitment and activation of the adaptor protein, FADD, and caspases, a family of cystein proteases that exist as inactive zymogens (any of a group of compounds that are inactive precursors of enzymes and are activated by a kinase) in normal cells (Balachandran et al., 2000). The cleavage of caspases, ultimately caspase-3, leads to the beginnings of apoptosis.

In normal cells, VSV is sensitive to the antiviral actions of alpha/beta interferons and the virus is destroyed. In many types of tumors these pathways are defective, allowing the virus to replicate. Replication of the virus occurs in the cyptoplasm of infected cells, which occurs independently of the cell cycle, and it preferentially kills cells that undergo mitosis (Marozin et al., 2010). In a normal cell, it is possible that IFN prevents apoptosis by blocking the early replication stages.

To determine whether it was a defective pathway that allowed this virus to be so effective, normal cells were treated with IFN-alpha and exposed to VSV and HCC tumor cells were exposed to VSV (Barber, 2004). Data indicated the normal cells were much more protected than the tumor cells. In the review done by Barber (2004), he plausibly indicated that following intravenous inoculation, VSV must infect a number of the animal's normal cells as well as the tumor cells. He stated that in this scenario, the IFN system would be activated within the normal cells and the virus replication thwarted. Secreted IFN from innocuously targeted cells, including high level IFN-producing plasmacytoid dendritic cells would activate anti-viral pathways in surrounding uninfected normal cells, causing them and the animal in general, to become resistant to VSV infection. In contrast to this, Barber (2004) stated that tumor cells would allow the replication of

VSV to proceed, due to their harboring defects in innate immune responses, and would lyse. Progeny viruses would, in turn, infect surrounding tumor cells and the process would begin again. This further supports the claim that it is the dysfunctional tumor cells that allow the virus to replicate and cause tumor shrinkage.

In earlier studies done by the group at Mt. Sinai, the wild type VSV, at levels similar to patient doses, induced a systemic proinflammatory cytokine response. This response might have been a contributing factor to lethal hepatotoxicity in the rats treated (Shinozaki et al., 2005). Signs of neurotoxicity include altered consciousness, excitability, and limb paralysis (Shinozaki et al., 2005). The wild type was also unable to establish an effective amount of tumor regression due to the host's immune response destroying the virus. In order for the virus to be successful, the host's immune response must first be suppressed. A recombinant version of the virus was created by this group that expressed equine herpes virus-1 glycoprotein G (rVSV-gG). This broad-spectrum viral chemokin binding protein was able to suppress the body's immune response. Although regression of the lesions in the liver were observed, complete tumor regression and long-term survival were not observed in the treated animals and they eventually relapsed (Wu et al., 2008). In the most recent study this group created another recombinant version of the virus that expressed M3, a chemokin-binding protein from murine gammaherpesvirus-68 (rVSV(MΔ51)-M3). Another benefit to this strain is the mutation in its matrix (M) at position 51, which results in an IFN-inducing phenotype (Wu et al., 2008). In this study, treated rats had a prolonged life with a 50% survival rate. Now they are looking at adding IFN-alpha to the treatment plan to better protect the normal cells and leave the tumor cells more susceptible to the virus. Hopefully, these two treatments working in conjunction can have a better, more positive result in the amount of tumor regression and prolonged survival rate in the rats and can advance to use in human medicine.

MATERIALS AND METHODS

The animal protocol for the hepatic artery injection is done by Marcia Meseck and follows the procedures as designated in the Handbook of Laboratory Animal Medicine. All sterile cotton swabs, gauze, and sponges are soaked in sterile phosphate buffered saline (PBS) solution. The rats are anesthetized using a VetEquip Isoflurane anesthesia chamber with the isoflurane level at 3 and oxygen level set at 2. The entire procedure is done in the biological safety cabinet and the rats are placed on sterile absorbent pads with their nose placed in the cone of the anesthesia machine. The hair is shaved on the abdomen and the skin is disinfected with 70% ethanol. A midline laparotomy (~3 cm long) is made and the left and right lobes of the liver and the intestines are gently moved to the exterior of the animal using a sterile, moistened cotton swab. The organs are covered with moistened gauze to prevent them from drying out. The membranes covering the liver lobes are cut to expose the common hepatic artery. The hepatic vessels (common hepatic artery, proper hepatic artery, and gastroduodenal artery) are gently exposed using a forceps and scissors without cutting any vessels or tissues. The gastroduodenal artery is ligated and the common hepatic artery was temporarily blocked using a micro clamp. The appropriate volume of the virus was slowly administered into the gatroduodenal artery. After the entire volume is administered, the proximal side of the gastroduodenal artery is ligated to prevent bleeding, the micro clamp is

removed from the common hepatic artery, and the presence of appropriate hepatic blood flow is confirmed. The intestines and liver lobes are returned to their physiological position. The abdominal incision is closed with sutures and the rat is placed back in its cage on its stomach. Each rat is also administered buprenorphine (analgesic) subcutaneously and ear tagged with the appropriate number.

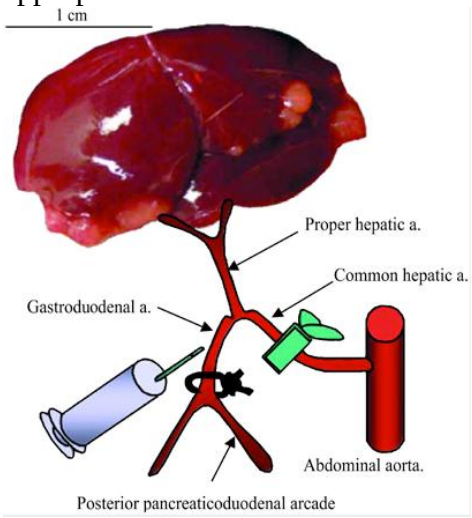

Figure 1. Hepatic Arterial Infusion of VSV in Multi-focal HCCbearing Syngeneic and Immune-competent Buffalo Rats

DATA

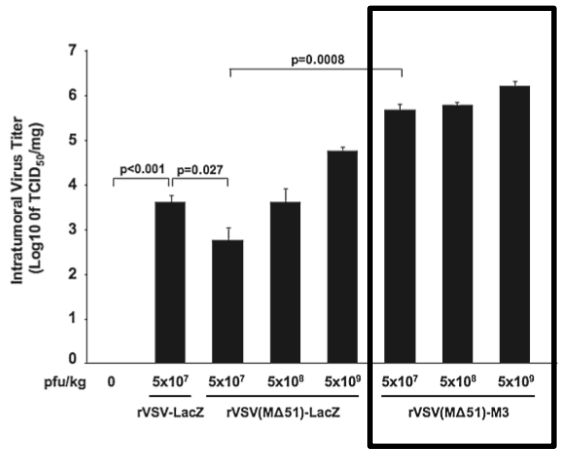

Figure 2. Multifocal HCC-bearing rats were injected with three different virus strains at different dosages. Rats were killed 3 days after virus adminstration. Viral titers show the virus most effective at staying in the body is the rVSV(MΔ51)-M3 strain (Wu et al., 2008).

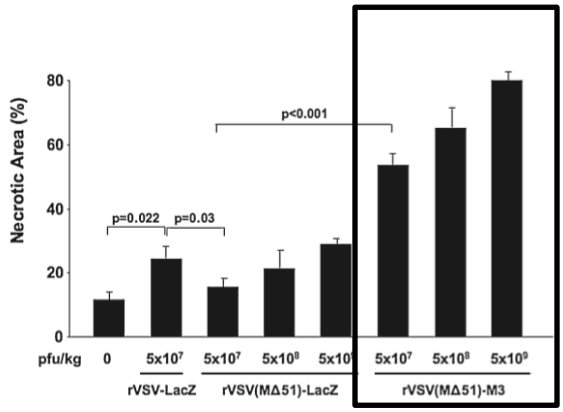

Figure 3. Tumor necrosis in rats treated with the same three strains of VSV. The percentage was the highest in the rVSV(MΔ51)-M3 strain and was determined by statistical analysis (Wu et al., 2008).

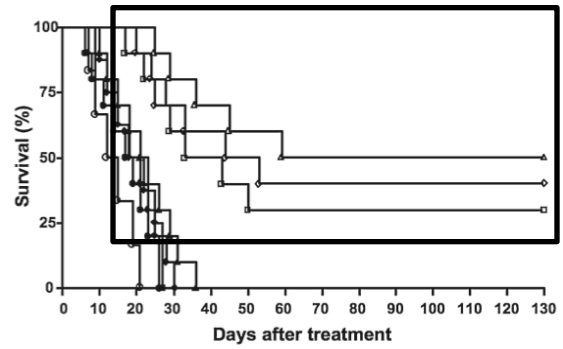

Figure 4. The box represent the rVSV(MΔ51)-M3 strain of the virus at the same three dosages and the other group of lines represent the other two strains used at the same dosages. Survival of the rats was observed daily and the highest survival percentage was seen in the rats given rVSV(MΔ51)-M3 (Wu et al., 2008).

RESULTS

Since the pathology results from this study will not be received for a couple of months, data was taken from the previous study with the recombinant virus rVSV(MΔ51)-M3 and speculations were made from these results with the addition of IFN-alpha. The results with the addition of IFN-alpha, should have an increased level of titers, necrosis, and survival since IFN-alpha should increase protection of the healthy cells, while leaving the tumor cells more susceptible to VSV. Hopefully, when the

pathology results come back that is the result.

LIMITATIONS

The main limitation of this study is the host's immune response. Every individual could respond to the introduction of VSV differently, despite the results concluded in the study. If an individual's innate immune system were particularly strong, this course of treatment for hepatocellular carcinoma would be ineffective. The probability of this occurring is difficult to predict and would most likely not occur, but nonetheless, it is always a possibility. The other extreme would be the patient's immune system is not strong enough to fight off the virus. In this case not only tumor cells would be targeted, but normal, healthy cells could be affected as well. Patient death may even result, as the recombinant version of the virus is able to suppress the immune system in order to do its job. An opportunistic infection that normally could be defended against, in the immune suppressed state of the host, could be harmful. Another limitation in the actual study is the time it takes to get the pathology results back. Speculations to whether or not the treatment was effective can be made, however, actual data to confirm those results has to wait for pathology.

IMPLICATIONS

The success of vesicular stomatitis virus as an oncolytic agent has prompted several other RNA viruses to be looked at for their oncolytic properties and some are even being used in clinical trials. Some of these viruses include reovirus, Newcastle disease virus, measles virus, vaccinia virus, and the influenza virus (Ausubel et al., 2011). Oncolytic viruses are now being looked at as treatment options for different cancers such as brain cancer and work has already begun to find a virus successful in the treatment of breast cancer. Many of these viruses that cause cancers, for example Hepatitis C is involved with Hepatocellular Carcinoma, have devised strategies to subdue the IFN/innate immune pathway to avoid destruction, so these cancers should be extremely susceptible to VSV (Balachandran et al., 2000; Barber, 2004). The idea that a virus, something our body naturally protects us against, can be beneficial to us is something that seems hard to believe, however, the prognosis so far seems good.

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